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NITRIFICATION ENHANCEMENT THROUGH PH CONTROL WITH
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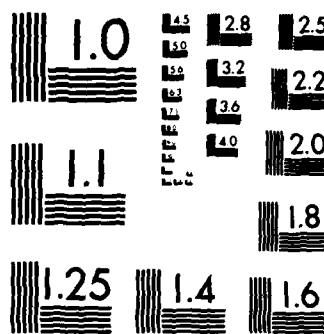
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NITRIFICATION ENHANCEMENT THROUGH PH CONTROL
WITH ROTATING BIOLOGICAL CONTRACTORS

David A. Long, Ph.D.

April 1982

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IMPROVEMENT OF NITRIFICATION IN ROTATING BIOLOGICAL
CONTACTORS BY MEANS OF ALKALINE CHEMICAL ADDITION



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INTRODUCTION

(Rotating Biological Contractor)

The need to achieve compliance with ammonia-nitrogen discharge limitations and the current emphasis on energy conservation have resulted in the utilization of RBC technology for the nitrification of secondary wastewater effluents. By the end of the 1970s, four pilot scale efforts, independent of the RBC industry, had been completed which demonstrated that the RBC could nitrify successfully secondary wastewater effluent (1, 2, 3, 4). In 1979, approximately 70 percent of the RBC systems in the United

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States were designed to remove carbonaceous biochemical oxygen demand (CBOD). Another 25 percent of the RBC systems were designed to remove CBOD and for nitrification in the same RBC units. The remaining 5 percent were constructed to nitrify secondary wastewater effluents in order to achieve ammonia-nitrogen effluent discharge limitations (5). Initial evaluations of full scale nitrifying RBC facilities reveal that they have not been completely satisfactory (6,7). Hittlebaugh (7) reported that an RBC facility, built for CBOD removal and nitrification, failed to meet design specifications during both winter and summer operations. The inability to meet CBOD and ammonia-nitrogen limitations during the summer was attributed to relatively low dissolved oxygen (DO) concentrations (less than 1 mg/l) in the initial nitrifying stages and a low pH (less than pH 7.0) in the latter nitrifying stages. The DO level increased during winter operations and CBOD was removed sufficiently to achieve design expectations. Ammonia-nitrogen removal also improved during the winter but not sufficiently to achieve design projections or effluent limitations. Recommendations from this study included the use of alkaline chemical feed systems to maintain optimum pH levels in order to improve nitrification.

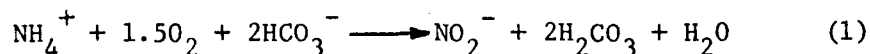
Nitrification within the RBC biofilm is essentially a two-step microbiological process which utilizes two groups of autotrophic bacteria of the family Nitrobacteraceae. The first group of bacteria oxidizes ammonia to nitrite and the second group of bacteria oxidizes nitrite to nitrate. The Nitrosomonas and Nitrobacter genera are considered to be the predominant nitrifying bacteria inhabiting the wastewater environment. Heterotrophic nitrification also occurs when nitrite or nitrate is produced from organic or inorganic compounds by heterotrophic organisms. Over 100 heterotrophic species (including fungi) have been identified which are capable of heterotrophic nitrification. However, the overall contribution to the oxidized nitrogen forms by heterotrophic nitrification is considered to be relatively small (8).

The growth rates of nitrifying bacteria are much slower than the growth rates of heterotrophic bacteria. This important distinction accounts for the inability of nitrification to proceed simultaneously with CBOD removal when high concentrations of organic material (greater than 30 mg/l of BOD) are present in the wastewater. Minimum doubling times reported for the ammonia-oxidizing bacteria are from 8 to 17 hours (9). Because the growth rates for nitrite-

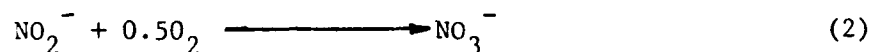
oxidizers are greater than the growth rates for ammonia-oxidizers (9,10), elevated nitrite concentrations normally do not persist and the ammonia-oxidation step controls the total amount of ammonia which is oxidized to nitrate within the wastewater environment. Carbon dioxide (CO₂) is the carbon source for these autotrophic nitrifying bacteria (11). Although some nitrifying bacteria have been observed to use organic compounds, they were not observed to utilize these organic compounds as the sole carbon source for growth (12). The generation of bacterial biomass per unit of ammonia oxidized (cell yield) is quite small. The total yield for both *Nitrosomonas* and *Nitrobacter* has been observed to be from 0.06 to 0.20 gram of cells per gram of ammonia oxidized (9). The nitrification of 20 mg/l of ammonia-nitrogen generates approximately 2 mg/l of solids (10). Therefore, the net amount of inorganic carbon required for this amount of nitrification is quite low. McGhee(13) reported that the inorganic carbon requirements for the nitrite oxidation step could be met without inorganic carbon being present in the bulk solution. The utilizable source of inorganic carbon was the CO₂ generated from endogenous respiration within the biofilm.

The simplified oxidative reactions below describe the salient aspects of the microbial oxidation of ammonia. The microorganisms derive energy from these reactions; this energy is used for CO₂ fixation.

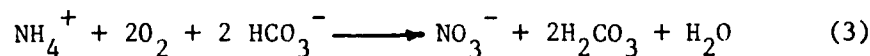
Ammonia Oxidation:



Nitrite Oxidation:



Overall Reaction:



As can be seen from Equation 1, the nitrification process results in the production of acid which neutralizes the alkalinity in the wastewater. Theoretically, 7.1 mg/l of alkalinity is destroyed for each 1 mg/l of ammonia oxidized. The destruction of alkalinity results in pH depression.

The actual pH depression is mitigated somewhat by the removal of carbonic acid through the stripping of CO_2 from the wastewater surface (10). However, under low alkalinity conditions, pH depression is enhanced due to the reduced buffer capacity of the wastewater. The level of alkalinity within wastewaters varies widely. The major factor influencing the amount of alkalinity present is the source of the carriage water, or the drinking water supply. High alkalinities normally are associated with ground water supplies and much lower alkalinities are associated with surface supplies. Domestic wastewater contributes from 50 to 200 mg CaCO_3/l to the natural alkalinity of the carriage water (14). Therefore, the amount of alkalinity in a domestic wastewater may range from less than 100 mg CaCO_3/l to several hundred mg CaCO_3/l . The net effect of such variations in alkalinity is to provide a different buffering capacity for each wastewater treatment system. Domestic wastewaters normally contain from 12 to 25 mg/l of ammonia-nitrogen. The range of alkalinity destroyed during the nitrification of these concentrations of ammonia is 85 mg CaCO_3/l to 178 mg CaCO_3/l . Obviously, the pH depression resulting from nitrification can be slight for low ammonia-high alkalinity wastewaters or significant for high ammonia-low alkalinity wastewaters. Wastewater pH levels are typically around pH 7.5. However, pH depression to below pH 7.0 is common for low alkalinity wastewaters.

The level of pH has an important effect on the nitrification process. There have been a number of researchers since the turn of the century who have addressed the subject of the effect of pH on nitrification. Those researchers who have made contributions pertinent to this study are listed in reverse chronological order in Table 1. It is interesting to note the large variation in the effect of pH on biological nitrification that is reported in the literature. The variation in the effect of pH on nitrification is due in large measure to the nature of the experiments undertaken, i.e., the homogeneity of culture involved (pure versus mixed culture); scale of the experiment (laboratory to full scale); nature of the biofilm (suspended versus fixed film) and a variety of (and frequently unspecified) acclimation times utilized in the experiments.

Several investigators (1, 3, 15, 16) within recent years have attempted to provide more information on nitrification within a fixed film mode. Haug and McCarty (15) utilized

Table 1. Literature Review of Optimum pH
Values for Nitrification.

Ref.	Author	Year	Optimum pH	Organism or System Studied
3	Miller, et al.	1979	8.0-8.5	RBC (Pilot)
1	Borchardt, et al.	1978	7.1-8.6	RBC (Pilot)
13	McGhee	1975	8.0-9.0	A.S. (Lab)
17	Srna & Baggaley	1975	7.45	Sub. Filt. (Lab)
18	Hutton & LaRocca	1975	8.4-8.6	A.S.
16	Huang & Hopson	1974	8.4-9.0	Biofilm (Lab)
15	Haug & McCarty	1972	7.8-8.3	Sub. Filt. (Lab)
19	Mulbarger	1972	8.4	A.S.
20	Wild, et al.	1971	8.4	A.S.
21	Loveless & Painter	1968	7.5-8.0	<u>Nitrosomonas</u>
22	Downing & Knowles	1967	7.2-8.0	--
23,24	Andersen	1964	8.4-8.5	<u>Nitrosomonas</u>
25	Boon & Landelot	1962	7.0-8.6	<u>Nitrobacter</u>
26	Engel & Alexander	1958	7.0-9.0	<u>Nitrosomonas</u>
27	Bushwell & Shiota	1954	8.0-8.5	<u>Nitrosomonas</u>
28	Hoffman & Lees	1952	8.0-9.0	<u>Nitrosomonas</u>
29	Meyerhoff	1917	8.5-8.8	<u>Nitrosomonas</u>
			8.5-9.0	<u>Nitrobacter</u>

a laboratory scale fixed film submerged reactor and a synthetic wastewater and performed a short term pH-nitrification study (18 hours at each pH value) using a biofilm developed at neutral pH and observed essentially the same rate of nitrification at pH 6.5 as at pH 9.0. At pH 6.0, the observed rate of nitrification was reduced to approximately 42 percent of the maximum rate and nitrification essentially stopped at pH 5.5. However, after only 10 days of operation at pH 6.0, the submerged filter was reported to have acclimated sufficiently to perform at the maximum rate of nitrification. This finding demonstrates the ability of nitrifying organisms to acclimate to low pH condition. The reason for this unique finding may be due in part to non-equilibrium conditions existing within the submerged filter after the startup period. Huang and Hopson (16) utilized a laboratory scale inclined fixed film surface and a synthetic wastewater to evaluate the effect of pH on nitrification. Their experiment examined the short term (less than 10 hours) effect of pH on the nitrification process and produced a maximum rate of nitrification at pH 8.4 to pH 9.0 with approximately 25 percent of the maximum rate occurring at pH 6.0. After three weeks of acclimation at pH 6.6, the rate of nitrification was approximately 85 percent of the maximum rate observed.

Borchardt (1) performed a short term pH-nitrification study utilizing a 0.6 meter pilot RBC treating domestic wastewater effluent from a trickling filter in a laboratory where ammonia, alkalinity and pH were controlled. The rate of nitrification was examined at eleven different levels of alkalinity after a short but undefined acclimation period. The results of this short term study revealed a nearly constant rate of nitrification between pH 7.1 and 8.6. Approximately 25 percent of the maximum rate of nitrification was observed at pH 6.5 and zero nitrification was indicated at pH 6.0. Borchardt was careful to point out the limitations of attempting to extrapolate his short term data into the long term.

Miller (3) most recently reported on a pilot scale 0.5 meter RBC treating domestic wastewater effluent from a pilot trickling filter wherein significantly greater rates of nitrification were observed at elevated pH levels (pH 8.0 to pH 8.5) than at neutral pH (approximately pH 7.1). This nitrification study is unique in that lime addition for phosphorus removal preceded the nitrification process and the nitrifying RBC stages had acclimated at

the elevated pH levels. A transition in biofilm performance was observed when the elevated pH of the wastewater was reduced to the neutral pH range. Nitrification performance initially remained unchanged. After approximately four days, the performance level started to deteriorate. In nine days, the performance had reverted to a lower nitrification level. This latter finding was not discussed fully by Miller; however, it is important because it helps to establish potential physical differences between the biofilms developed at neutral and elevated pH levels. This situation was not observed by the other investigators using fixed films mentioned above because none ever attempted to acclimate biofilms at the elevated pH levels. Such differences cannot be assumed to be purely indicative of only the pH dependent rates of microbial nitrification. These differences also are reflective of the entire heterogeneous population developed within each biofilm which dictate film development, cohesion, and retention characteristics (sludge age). There is essentially no information within these wastewater nitrification studies which addresses changes in biofilm and microbial populations under various pH conditions. In general, this important consideration has been ignored in such wastewater research studies. However, current research efforts such as those by Olem (30), LaMotta (31) and Characklis (32) are starting to examine more closely the mechanics of biofilm development and the characterization of microbial populations.

The addition of alkaline chemicals to wastewater treatment systems to increase pH and provide added buffer capacity has been attempted with varying degrees of success. Heidman (33) conducted a pilot study at the Blue Plains WWTP using an activated sludge system which incorporated pH controlled nitrification. This study was inconclusive because it failed to demonstrate the relative nitrification without chemical addition. Hutton (18) demonstrated the feasibility of optimizing the nitrification of high ammonia strength industrial wastewaters with alkaline chemical addition. Lue-Ling (34) reported success in using alkaline chemical addition to nitrify high ammonia strength lagoon supernatant with RBCs. Hittlebaugh (35) attempted to enhance the nitrification of domestic wastewater with RBCs through alkaline chemical addition; however, the results were inconclusive. The literature fails to address the efficacy of optimizing domestic wastewater

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1. Establish the relative rates of nitrification for domestic wastewater treatment within an acclimated RBC fixed film system as a function of pH.
2. Observe and characterize the relative changes in the RBC biofilm as a function of pH.
3. Evaluate the efficacy of chemical addition to improve nitrification within an RBC fixed film system through the maintenance of an optimum pH.
4. Evaluate alternative alkaline chemicals for pH controlled nitrification for the RBC.
5. Develop design criteria, as appropriate, for pH controlled nitrification for the RBC.

EXPERIMENTAL PROCEDURES

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The effect of alkaline chemical addition was evaluated utilizing five 2-stage RBCs operating in parallel (Figure 2). The level of nitrification of a low pH 2-stage control RBC system (control) was compared against the nitrification level of four other 2-stage RBC systems receiving four different alkaline chemicals. The four alkaline chemicals used were calcium hydroxide, sodium carbonate, sodium hydroxide, and sodium bicarbonate.

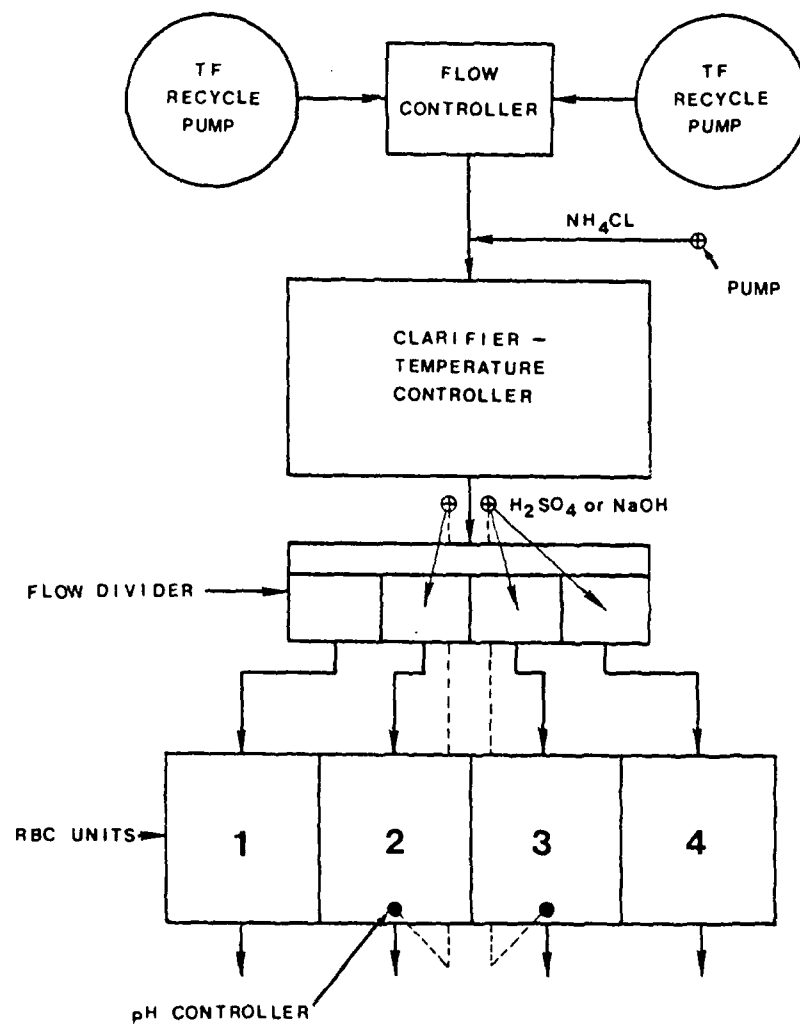


Figure 1. Schematic Diagram of the Pilot RBC Units for the Low pH- and High pH-Nitrification Study

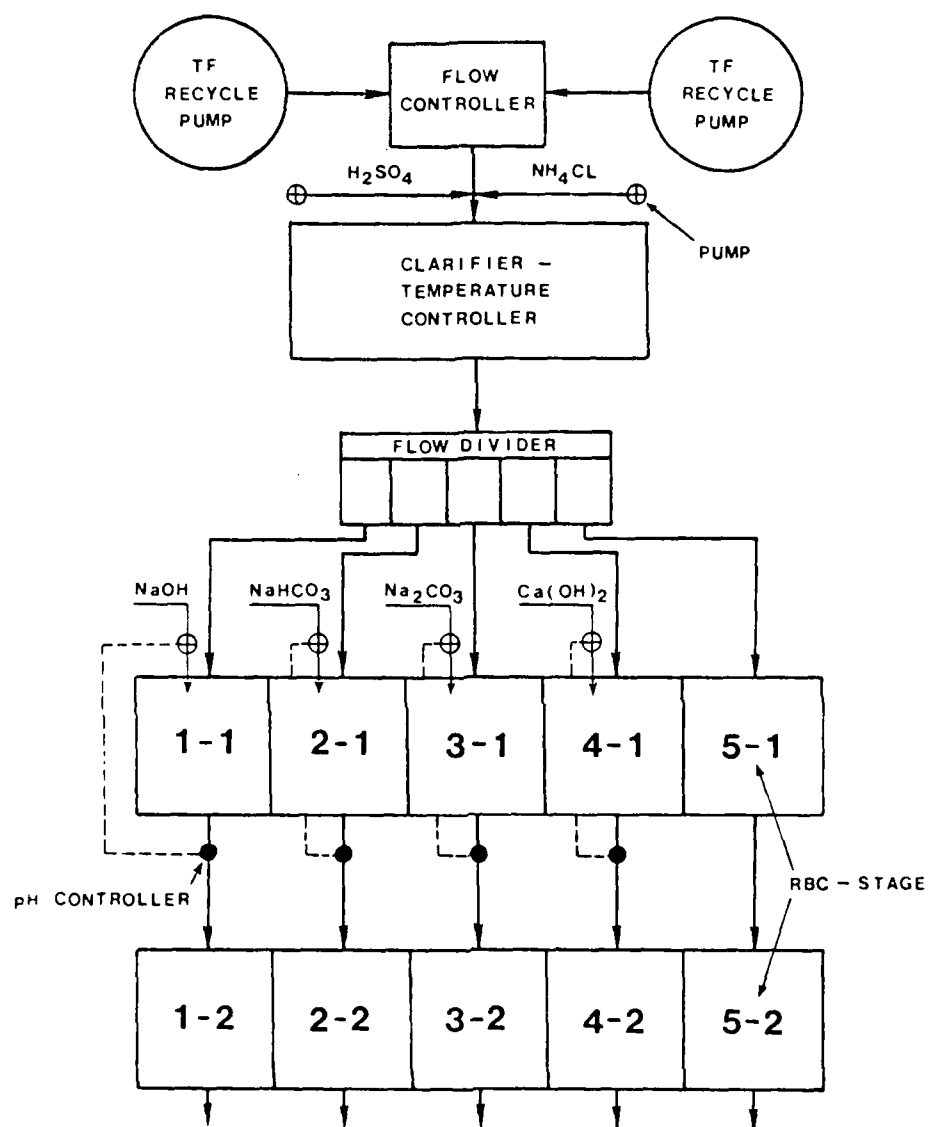


Figure 2. Schematic Diagram of the 2-Stage RBC Systems of the Alkaline Chemical Addition Study.

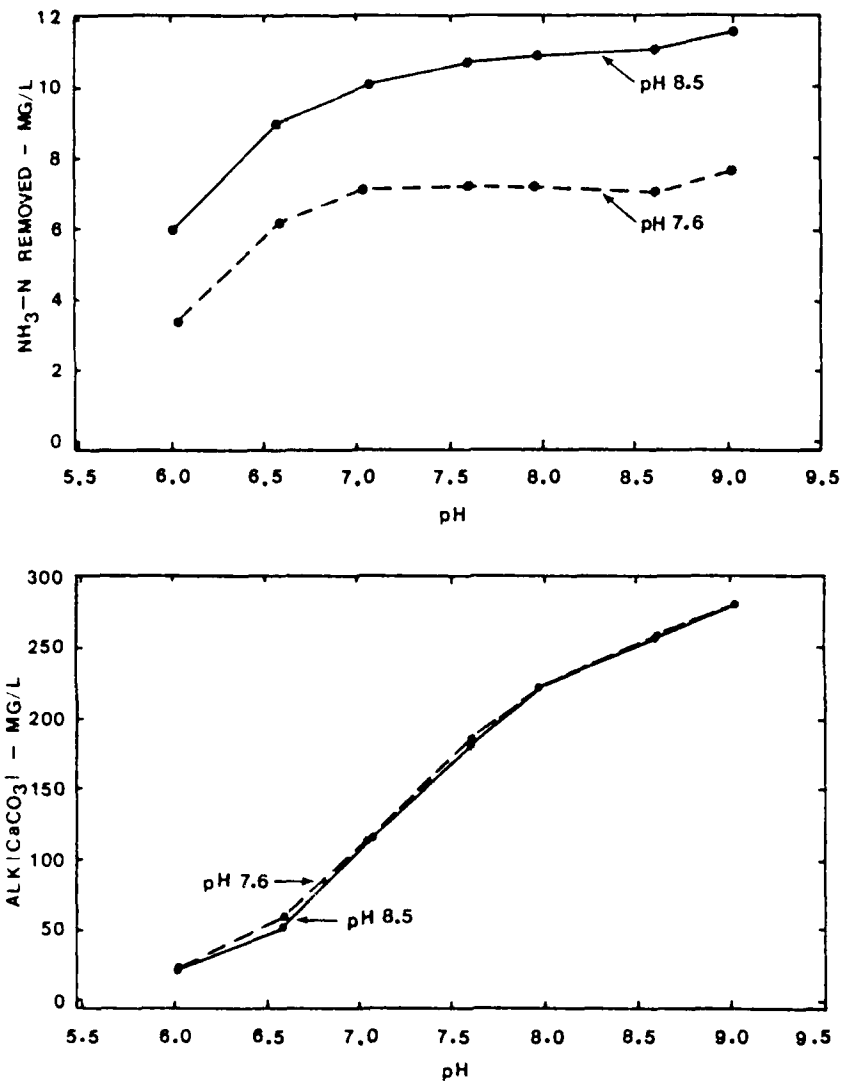
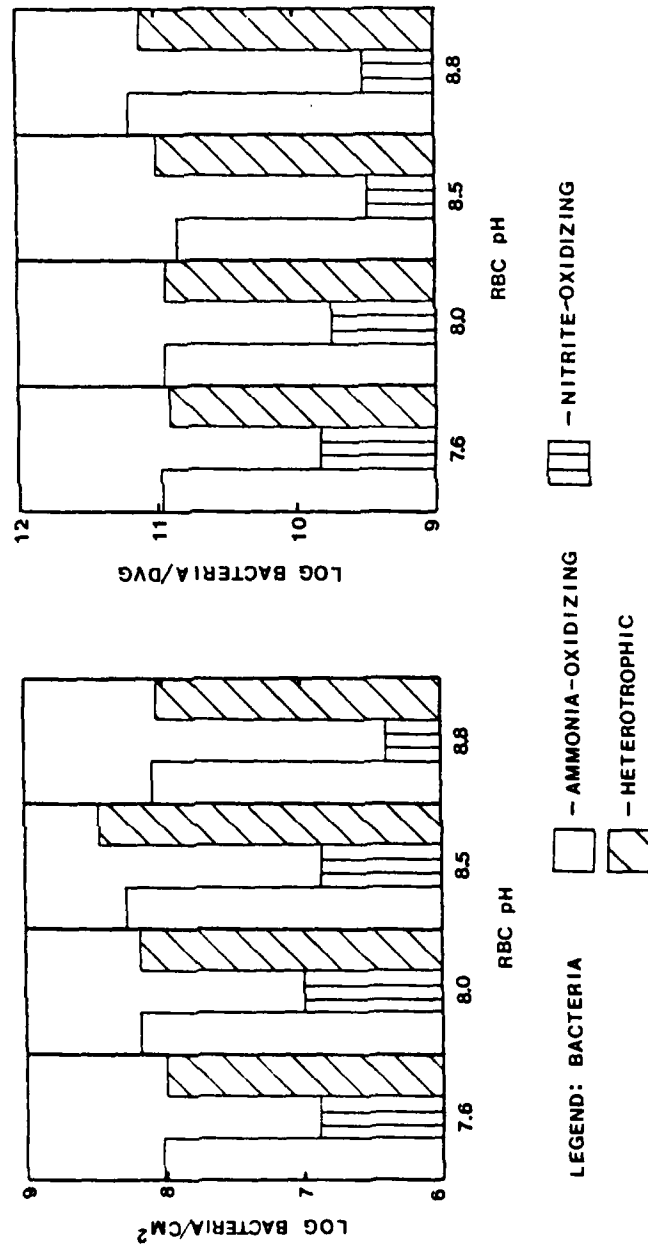


Figure 7. The Relative Rates of Nitrification of RBC Systems Acclimated at pH 8.5 and pH 7.6 and Subjected to Short Term pH Changes and Related Alkalinity Levels

the pH 7.6, 8.0, and 8.5 systems but greatest at pH 8.8. Similarly, the heterotrophic population increased with pH. However, the nitrite-oxidizing bacteria populations were nearly identical at pH 7.6 and pH 8.0 but lower at pH 8.5 and pH 8.8. The ratios of heterotrophic to ammonia-oxidizing to nitrite-oxidizing bacteria based on the data presented in Figure 6 for the pH 7.6, 8.0, 8.5, and 8.8 RBC units were 12:15:1, 16:16:1, 37:24:1, and 43:48:1, respectively. In general, these population figures indicate that the heterotrophic bacteria and the ammonia-oxidizing bacteria are favored over the nitrite-oxidizing bacteria with respect to increasing pH. During this period, the mean biofilm concentrations were 1.80, 2.26, 2.99, and 1.23 mg/cm² for the pH 7.6, 8.0, 8.5, and 8.8 RBC units, respectively. The volatile content was 86, 86, 83, and 75 percent for the pH 7.6, 8.0, 8.5, and 8.8 RBC units, respectively. This lower volatile content of the pH 8.8 RBC was attributed to low level precipitation of calcium carbonate and entrainment of the precipitate within the disc biofilm.

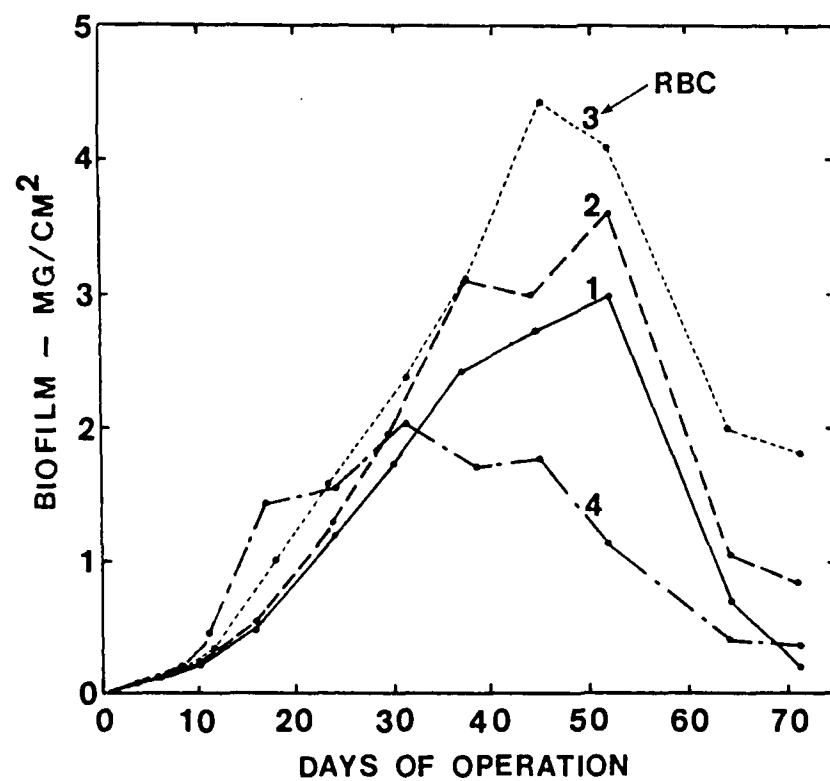
At the conclusion of the 10-week high pH-nitrification study, the two RBC systems which had been operating at pH 7.6 and pH 8.5 were utilized in a short-term pH-nitrification study wherein the two RBC systems experienced simultaneous short term changes in pH, i.e. 2 hours of operation at each pH level. The pH level started at pH 9.0 and was decreased progressively downward to pH 6.0 without interruption. Alkalinity and pH levels were maintained in each RBC by direct feed of sodium hydroxide and sulfuric acid solutions. This test was run twice (Day 73 and Day 79) with similar results. The average values of nitrogen removal data obtained from these two runs are shown in Figure 7.

The shapes of the performance curves for the two RBCs are similar yet the level of nitrification for the two RBCs are markedly different. Clearly, the RBC which had acclimated at pH 8.5, and had a history of elevated performance, retained its higher performance level in the short-term and continued to perform significantly better than the biofilm acclimated at pH 7.6. The RBC response to short-term changes in pH is relatively constant between pH 7.9 and pH 9.0 but highly dependent upon the previous acclimated level of nitrification for the given RBC biofilm. Data on the alkalinity levels also have been included in Figure 7. The amount of alkalinity present at the low



NOTE: Geometric means are based upon six sets of enumerations for each bacteria classifications from Day 30 to Day 71. Nitrifying enumerations are MPN values and heterotrophic enumerations are plate counts.

Figure 6. Relative Geometric Mean Populations of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria on a Unit Area and Unit Weight of Dry Volatile Disc Bio-film Basis for the RBCs of the High pH-Nitrification Study



LEGEND: RBC ——— 1-pH 7.6 - - - - 2-pH 8.0
 ····· 3-pH 8.5 - · - · 4-pH 8.8

Figure 5. RBC Disc Biofilm Development for the High pH-Nitrification Study

operation. By the eighth day, the four RBCs had developed thin and highly uniformly textured biofilms which possessed a visually apparent gradation. The pH 8.5 and pH 8.8 RBC biofilms initially developed more rapidly than did the pH 7.6 and pH 8.0 RBC biofilms. This initial biofilm gradation was not related to ammonia removal efficiency. The biofilm color had changed from reddish-brown to tan or bronze on all discs by the 10th day. By the 13th day of operation, the two lower pH RBCs had the most uniformly textured biofilms while the pH 8.5 and pH 8.8 RBCs were developing a "dimpled" appearance associated with the heavier biofilms. The pH 8.8 RBC developed a patchy appearance and also had started to slough significantly after only two weeks. As time progressed, the RBC systems added biofilm, but their texture became less uniform. The heavier biofilms appeared to be associated with the pH 8.0 and pH 8.5 systems. The pH 8.8 RBC experienced the greatest biofilm sloughing. Figure 5 presents the RBC disc biofilm development data for all four RBC systems during the high pH-nitrification study. After the initial month of operation, the levels of disc biofilm for the pH 7.6, 8.0, and 8.5 RBCs were related directly to their relative level of performance. The pH 8.8 RBC experienced an initially high rate of biofilm development; however, it reached a peak mass per unit area concentration on Day 31 and then experienced a continuous biofilm loss. All four RBC systems experienced a marked decline in disc biofilm after the end of the PSU spring term on Day 52 when the influent CBOD concentration decreased. The four RBC systems appeared to achieve an initial maximum level of nitrification performance in about three weeks. These performance levels corresponded to disc biofilm concentrations of approximately 0.8, 1.0, 1.2 and 1.4 mg/cm² for the pH 7.6, 8.0, 8.5, and 8.8 RBCs, respectively. As demonstrated previously, the increase in disc biomass did not improve the rate of nitrification for any of the systems.

The data on the relative geometric mean bacteria populations per unit of disc area and per unit weight of dry volatile biofilm for each RBC system after the initial 30 days of operation are presented graphically in Figure 6. The total numbers of ammonia-oxidizing and heterotrophic bacteria increased with increasing pH up to pH 8.5 and then experienced a drop at pH 8.8. The total number of nitrite-oxidizing bacteria was greatest at pH 8.0 and decreased at pH 8.5 and pH 8.8. The number of ammonia-oxidizing bacteria relative to the total biofilm population was similar for

and the pH 8.0 RBC removed 94 and 84 percent as much ammonia as did the pH 8.5 system, respectively; whereas the control RBC removed only 65 percent as much ammonia. Data on the nitrogen balances for the four RBC systems for this time period are presented in Table 6. As noted earlier, the slightly lower nitrogen recoveries at the higher pH conditions may be due to small nitrogen losses resulting from ammonia stripping and denitrification within the heavier biofilms. These nitrogen recovery results indicate that ammonia stripping was not a major factor affecting the change in ammonia levels between pH 7.6 and pH 8.8.

Table 6. RBC Nitrogen Balances for the High pH Nitrification Study^a

RBC	pH	Total Nitrogen ^b Influent - mg/l	Effluent	Percent Recovery
1	7.6	21.7(21) ^c	21.1(21)	97
2	8.0	21.5(21)	20.2(21)	94
3	8.5	21.7(21)	20.6(21)	95
4	8.8	21.8(21)	20.8(21)	95

^aNitrogen balances are based upon data from Day 38 to Day 71.

^bTotal nitrogen is total oxidized nitrogen plus total Kjeldahl nitrogen (TKN).

^cNumber in parenthesis is the number of samples utilized in the total nitrogen evaluations.

The four RBCs developed biofilms which could be sensed by touch within 48 hours. A noticeable reddish-brown biofilm was evident on all the discs by the third day of

lower pH RBC units. The volatile contents for these biofilms were 82, 83, 84, and 81 percent for the pH 7.5, 7.1, 6.5, and 6.3/6.7 RBCs, respectively.

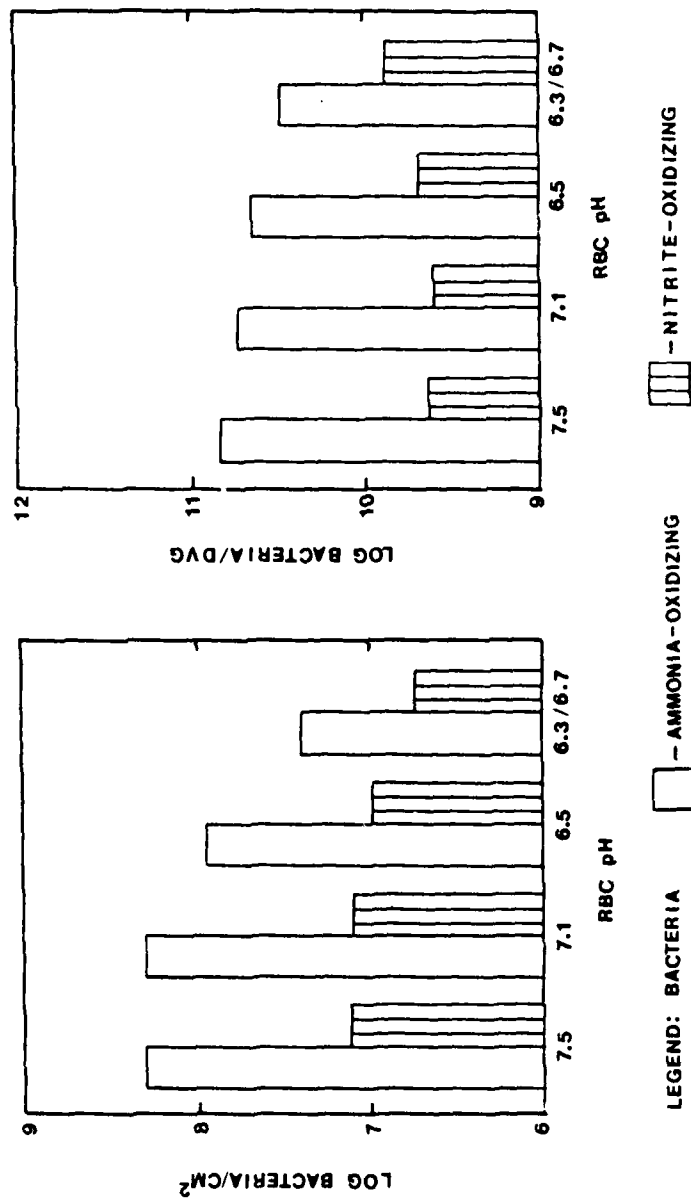
High pH-Nitrification Study

This research phase was devoted to the evaluation of the relative rates of nitrification in single stage RBC systems operated at pH 7.6, 8.0, 8.5, and 8.8. The pH 7.6 RBC treated the unaltered wastewater and served as the control. The operational characteristics of the four RBC systems were the same as reported previously in Table 2. On Day 36, a pH excursion to approximately pH 11.0 for an estimated two hours occurred within the pH 8.8 RBC with rather dramatic results. This short-term transient condition appeared to have little effect on the ammonia-oxidation process. However, the RBC experienced an immediate loss of nitrite-oxidation capability and a very slow nitrite-oxidation recovery. Based upon ammonia removal, and not complete oxidation, a period of relative equilibrium was established after approximately five weeks of operation. Data on the relative amounts of ammonia-nitrogen removed by the RBCs are presented in Table 5. The pH 8.8 RBC

Table 5. Relative Rates of Nitrification for RBC Systems Operating Under High pH Conditions^a

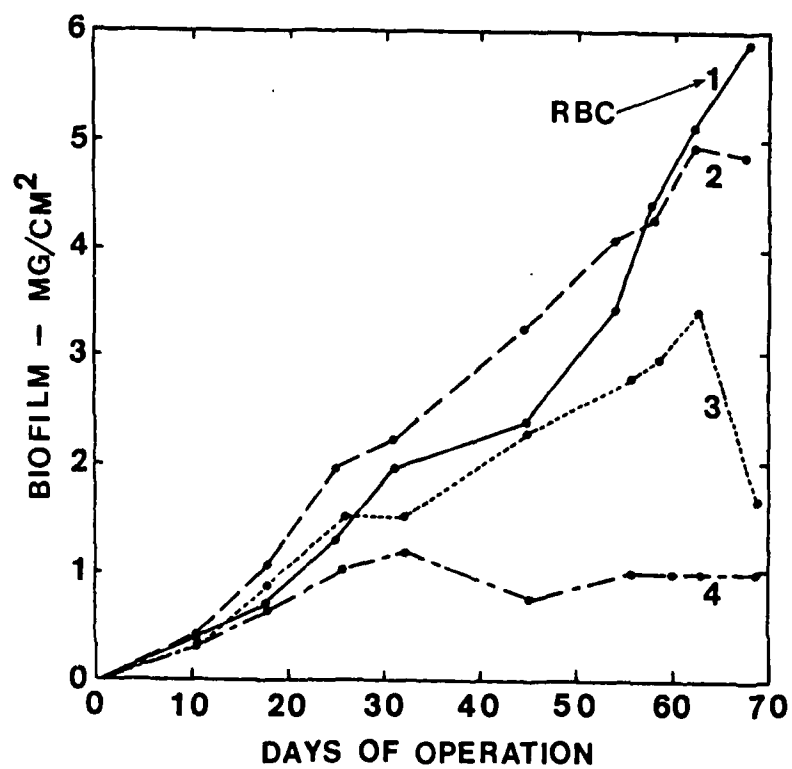
RBC pH	Ammonia-N Removed (g NH ₃ -N/m ² ·d)	Percent of Maximum
7.6	2.0	65
8.0	2.6	84
8.5	3.1	100
8.8	2.9	94

^aBased upon data from Day 38 to Day 71



NOTE: The MPN data are geometric means of weekly enumerations starting after Day 30 and ending on Day 69.

Figure 4. Relative Geometric Mean Populations of Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria on a Unit Area and Unit Dry Volatile Weight Basis for the Low pH-Nitrification Study



LEGEND: RBC — 1-pH 7.5 --- 2-pH 7.1
 3-pH 6.5 -.- 4-pH 6.3/6.7

Figure 3. RBC Disc Biofilm Development for the Low pH-Nitrification Study

patchy appearance was attributed mainly to biofilm loss resulting from hydraulic shear. However, biofilm sloughing from the disc surface did occur.

The RBC disc biofilm development data for all four RBC systems during the low pH-nitrification study are presented in Figure 3. The RBC systems at pH 7.5 and 7.1 showed the best performance and had the most disc biofilm. The pH 6.5 RBC showed a lower level of performance and less biofilm. The RBC which was operated initially at pH 6.3 and later adjusted to pH 6.7 had the lowest performance level throughout most of the 69-day study and also developed the least amount of biofilm. The maximum ammonia-oxidation levels for the pH 7.5, 7.1, and 6.5 RBC's were achieved when the biofilm masses were approximately 2.0, 2.2, and 1.5 mg/cm² respectively. Increases in disc biomass did not enhance the nitrification rates for any of these RBC systems. The pH 6.3/6.7 RBC added biofilm during the first three weeks of operation at a rate comparable to that of the pH 7.5 RBC yet showed no nitrification capacity. This result indicates that, at least initially, organisms other than nitrifying bacteria were inhabiting the RBC discs.

The relative geometric mean data for the nitrifying bacterial populations per unit disc area and per unit volatile weight for each RBC system after the initial month of startup are presented in Figure 4. These graphs demonstrate clearly that the total number of viable nitrifying bacteria on each RBC was related directly to overall RBC nitrification performance. The higher pH systems had larger populations of both ammonia-oxidizing and nitrite-oxidizing bacteria. The sustained depressed nitrification performance and the relatively low nitrifying bacteria populations of the 6.3/6.7 RBC indicated that a significant period of time was required for complete autotrophic adjustment in response to system changes under relatively low pH conditions. The ratios of ammonia-oxidizing bacteria to nitrite-oxidizing bacteria for the pH 7.5, 7.1, 6.5 and 6.3/6.7 RBC units were 16:1, 14:1, 9.4:1 and 3.3:1, respectively. This observation indicates that the lower pH systems favor nitrite-oxidizing bacteria relative to ammonia-oxidizing bacteria. This conclusion is shown graphically in Figure 4 which shows that the number of ammonia-oxidizing bacteria per dvg (dry volatile gram) decreased with decreasing pH, while the number of nitrite-oxidizing bacteria per dvg increased for the two

Table 4. RBC Nitrogen Balances for the
Low pH-Nitrification Study^a

RBC	pH	Total Nitrogen ^b Influent	- mg/l Effluent	Percent Recovery
1	7.5	19.5(23) ^c	19.3(23)	99
2	7.1	19.6(23)	19.7(23)	100
3	6.5	19.7(24)	20.3(24)	103
4	6.3/6.7	19.6(23)	20.2(23)	103

^aBased upon data from Day 37 to Day 69

^bTotal nitrogen is total oxidized nitrogen plus total Kjeldahl nitrogen (TKN)

^cNumber in parenthesis is the number of samples utilized in the total nitrogen determinations.

The four RBCs developed biofilms which could be sensed by touch within 48 hours. A noticeable bronze color developed after five days of operation. By the tenth day, all RBC systems had developed thin and highly uniformly textured coatings which possessed a visually apparent gradation. The heaviest biofilm appeared in the pH 7.5 RBC and the lightest growth of biofilm was in the pH 6.3/6.7 RBC. All four RBC units showed some degree of sloughing by Day 19 with the greatest sloughing occurring in the pH 6.3/6.7 RBC. The biofilm color changed from bronze to brown with increasing age and increased biomass. After the loss of the initial biofilm uniformity, the biofilm became increasingly patchy with time and decreasing pH. At the conclusion of this phase of the study, the non-uniformity of the biofilm was quite evident visually and seemed to be related directly to the relative nitrification rates recorded over the duration of the study. The

during the first 25 days of operation. Two short-term pH excursions may have had an adverse impact on nitrification development on the pH 6.3 RBC. On Day 27, the pH of the pH 6.3 unit was adjusted upward to pH 6.7 in an attempt to obtain additional information regarding the nitrification rate between pH 6.5 and pH 7.1; this RBC is referred to hereafter as the 6.3/6.7 RBC. Based upon nitrification performance, a period of relative equilibrium was established by about Day 37 for the pH 7.5, 7.1, and 6.5 RBC units. Data on the relative amounts of ammonia-nitrogen removed by the RBCs are presented in Table 3. The rate of nitrification of the pH 7.1 RBC system was 96 percent of that observed at pH 7.5 and the rate for the pH 6.5 RBC was 80 percent of the rate for the pH 7.5 RBC. Because of the long period of time required for nitrification to become established in the pH 6.3/6.7 RBC, data for this unit are not included in Table 3. Nitrogen balances for each

Table 3. Relative Rates of Nitrification for RBC Systems Operating Under Low pH Conditions^a

RBC pH	Ammonia-N Removed g NH ₃ -N/m ² ·d	Percent of Maximum
7.5	2.5	100
7.1	2.4	96
6.5	2.0	80

^aBased upon data from Day 37 to Day 69

of the RBC systems are presented in Table 4. The slightly lower nitrogen recoveries for the two higher pH systems might reflect small nitrogen losses associated with denitrification within the heavier biofilms as well as minor losses due to ammonia stripping.

Table 2. Pilot Single-Stage Nitrifying
RBC Operating Characteristics

Secondary Clarifier

Surface Settling Rate (@6.8 m ³ /d)	- 5.9 m ³ /d·m ²
Detention Time (@6.8 m ³ /d)	- 2.1 hr

RBC

Number of RBCs	- 4
Stages per RBC	- 1
Discs per Stage	- 9
Disc Diameter	- 0.5 m
Disc Area - Total	- 5.3 m ²
Rotational Speed	- 13 rpm
Peripheral Speed	- 0.34 m/sec
Hydraulic Loading ^a	- 81 l/m ² ·d

^aThe hydraulic loading for all four RBC units was nominally 81 l/m²·d (2 gal/d·ft²). The hydraulic loading calculation is based upon the assumption that each RBC is the first stage of a 4-stage RBC.

The alkaline chemicals were added to the first stage only. The first stages of the calcium hydroxide, sodium carbonate, and sodium hydroxide RBC systems were maintained at the optimum pH level for nitrification (approximately pH 8.5). The first stages of the sodium bicarbonate and the control RBC systems were maintained at pH 7.5 and pH 7.0, respectively. The low pH wastewater was created by adding sulfuric acid prior to clarification and the alkaline chemicals then were added directly to the first stage of each RBC. Ammonium chloride was added to the wastewater during PSU break periods to augment the low influent ammonia-nitrogen. There was no biomass on the discs at the start of the test. The observation period for this part of the study lasted approximately 11 weeks.

Wastewater sampling was accomplished by compositing grab samples on influents and effluents. Biofilm sampling and analyses were performed in accordance with modifications of the procedures reported by Olem (30). The most probable numbers (MPN) of ammonia-oxidizing bacteria were determined using a modification of the Nitrosomonas MPN technique of Alexander and Clark (36) which was reported by LaBeda and Alexander (37) as well as Rowe (38). The nitrite-oxidizing bacteria MPN values were determined using a modification of the Nitrobacter MPN technique of Alexander and Clark (36) which was reported by LaBeda and Alexander (37) and Ghiorse and Alexander (39). The enumeration of heterotrophic bacteria was accomplished by spread plating serial dilutions of Modified Taylor's Media (40). Detailed descriptions of all sampling and analytical procedures are found in Stratta (41).

RESULTS AND DISCUSSION

Low pH-Nitrification Study

This research phase was devoted to the evaluation of the relative rates of nitrification in single stage RBC systems operated at pH 7.5, 7.1, 6.5 and 6.3. The pH 7.5 RBC treated the unaltered wastewater and served as the control. Data showing the operational characteristics of the four RBC systems are presented in Table 2. The rates of nitrification which initially developed for the pH 7.5 and the pH 7.1 RBC systems were greater than the nitrification rates of the two lower pH RBC systems. Nitrification was not established in the pH 6.3 RBC system

pH levels demonstrates that even at 20 mg/l of CaCO_3 , significant amounts of nitrification were achieved.³ The response of the lower pH system closely resembles the data of Borchardt (1) and similarly reveals good nitrification at very low alkalinity levels. This result tends to reinforce the observation that the pH level is much more important than the alkalinity level per se in terms of effect on nitrification. The amount of time required for an RBC system to adjust to an altered pH condition is discussed further in Stratta (41).

Alkaline Chemical Addition Study

This phase of the research was devoted to the evaluation of the rates of nitrification of 2-stage RBC systems maintained at elevated pH levels through alkaline chemical addition. The pH and alkalinity levels within the first stages of four RBC systems were adjusted upward and maintained artificially with four different alkaline chemicals. Calcium hydroxide, sodium carbonate, and sodium hydroxide were used to maintain approximately pH 8.5, the optimum pH, in the first stage of three different RBCs. Sodium bicarbonate was used to maintain pH 7.5 in the first stage of the fourth RBC. A fifth RBC which treated low pH-low alkalinity wastewater was used as a control. Data on the operational characteristics of the five RBC systems are presented in Table 7.

The overall nitrification capacity of the 2-stage RBC control system developed more slowly than did that of the higher pH systems. However, the control RBC and the alkaline chemical feed RBC systems were operating at approximately the same level of nitrification performance after a little more than three weeks of operation. The levels of performance for all five systems were very similar for about the next ten days. The overall performance of the control system, operating at the lower pH level, started to deteriorate after approximately 35 days of operation.

Based upon the overall nitrification performance of the five 2-stage RBC systems, a period of relative equilibrium was established by about Day 38. The data on the relative amounts of total ammonia-nitrogen removed by the five RBCs from Day 38 to Day 75 are presented in Table 8. These data show that the overall ammonia-nitrogen removals for the sodium hydroxide, sodium carbonate, and

Table 7. Pilot 2-Stage Nitrifying RBC
Systems' Operating Characteristics

Secondary Clarifier

Surface Settling Rate (@ 8.6 m ³ /d) -	7.4 m ³ /d·m ²
Detention Time (@ 8.6 m ³ /d) -	1.7 hr

RBC

Number of RBCs -	5
Stages per RBC -	2
Discs per Stage -	9
Disc Diameter -	0.5 m
Disc Area - Total -	10.6 m ²
Rotational Speed -	13 rpm
Peripheral Speed -	0.34 m/sec
Hydraulic Loading ^a -	81 l/m ² ·d

^aThe hydraulic loading for all five RBC units was nominally 81 l/m²·d (2 gal/d·ft²). The hydraulic loading calculation is based upon the assumption that each 2-stage RBC system contained the first two stages of a 4-stage RBC.

Table 8. Relative Rates of Nitrification for the RBC Systems of the Alkaline Chemical Addition Study^a

RBC	Alkaline Chemical	Ammonia-N Removed (g NH ₃ -N/m ² ·d)	Percent Removed	Percent of Maximum
1	NaOH	2.52	86	99
2	NaHCO ₃	2.40	82	94
3	Na ₂ CO ₃	2.54	87	100
4	Ca(OH) ₂	2.55	87	100
5	Control	2.14	73	84

^aBased upon data from Day 38 to Day 75

calcium hydroxide RBC systems were nearly the same. The performance level of the sodium bicarbonate RBC system was about 6 percent less than that of the other three high pH systems. The control RBC, which was operated at the lowest pH conditions, removed about 16 percent less ammonia-nitrogen than did the three high pH alkaline chemical feed systems. Table 9 presents the ammonia removal data for each respective RBC stage. The data on the amounts of ammonia-nitrogen removed clearly demonstrate that the greatest removal occurs at the elevated pH conditions. The amount of ammonia removed by the first and second stages of the three high pH alkaline chemical feed RBC systems was essentially the same. The sodium bicarbonate RBC system had lower pH levels and lower performance in both stages. The control had the lowest stage pH levels and the poorest performance for both stages. Nitrogen balances during this period for the stages of the five RBC systems are presented in Table 10. These nitrogen balances follow the same pattern previously reported. The percent recovery decreased slightly as pH increased. Again, this lower

Table 9. Relative Rates of Nitrification for the Stages of the RBC Systems of the Alkaline Chemical Addition Study^a

RBC-Stage	Alkaline Chemical	pH	Ammonia-N Removed (g NH ₃ -N/m ² ·d)	Percent Removed	Percent of Maximum
1-1	NaOH	8.5	2.53	43	98
2-1	NaHCO ₃	7.5	2.33	40	91
3-1	Na ₂ CO ₃	8.4	2.55	44	99
4-1	Ca(OH) ₂	8.5	2.57	44	100
5-1	Control	7.0	2.14	37	83
1-2	-	7.9	2.50	77	98
2-2	-	7.7	2.46	72	97
3-2	-	8.0	2.52	78	99
4-2	-	7.9	2.54	78	100
5-2	-	6.9	2.14	59	84

^aBased upon data from Day 38 to Day 75

^bBased upon ammonia-nitrogen influent to each RBC stage.

^cBased upon maximum ammonia-nitrogen removed by calcium hydroxide RBC stages.

Table 10. RBC Nitrogen Balances for the Alkaline Chemical Addition Study^a

RBC-Stage	Alkaline Chemical	pH	Total Nitrogen ^b mg/l	Percent Recovery	
				Stage ^d	RBC
INFLUENT	-	6.5	24.8(24) ^c	-	-
1-1	NaOH	8.5	24.4(26)	98	-
2-1	NaHCO ₃	7.5	24.0(24)	97	-
3-1	Na ₂ CO ₃	8.4	23.5(25)	95	-
4-1	Ca(OH) ₂	8.5	23.7(25)	96	-
5-1	Control	7.0	24.4(25)	98	-
1-2	-	7.9	22.8(24)	93	92
2-2	-	7.7	23.0(25)	96	93
3-2	-	8.0	22.3(25)	95	90
4-2	-	7.9	22.3(25)	94	90
5-2	-	6.9	23.8(25)	98	96

^aBased upon data from Day 38 to Day 75.

^bTotal nitrogen is total oxidized nitrogen plus total Kjeldahl nitrogen (TKN).

^cNumber in parenthesis is the number of samples utilized in the total nitrogen determinations.

^dNitrogen balances for the stages are based upon stage influent nitrogen.

recovery is attributed to small ammonia losses due to ammonia stripping and denitrification within the thicker biofilms associated with the higher pH levels.

Biofilm which could be sensed by touch had developed on the discs of all stages within 36 hours. Within 72 hours from startup, all first stage discs had developed biofilms which were noticeably heavier than the second stage biofilms. The characteristic tan color associated with nitrifying biofilms had developed by Day 4 and was more apparent in the first stage biofilms. All of the biofilms were very uniform in texture. This initial biofilm growth appeared to be heavier than the biofilms developed during the previous research phases. By Day 6, the trough walls also had developed noticeable amounts of biofilm. Although both stage biofilms got heavier and darker with time, the heavier and darker biofilms were on the first stage discs. The first stage biofilms became brown while those on the second stage discs remained tan to bronze in color. By Day 16, discs in all the first stages had experienced some sloughing while those in the second stages retained their uniformity. The loss of uniformity in the second stages commenced about Day 21. As time progressed, the loss of biofilm uniformity was greatest for the control and the sodium bicarbonate RBC systems. The first stages of the calcium hydroxide, sodium carbonate, and the sodium hydroxide RBC systems had the heaviest and most uniform biofilm coatings. The biofilm uniformity related directly to the ammonia removal performance levels of the RBC systems. The loss of biofilm during the 75-day period was attributed mainly to hydraulic shear starting on the surface of the biofilm and progressing inward. Biofilm sloughing from the bare disc outward did not occur continuously. The former method of sloughing appeared to be associated with relatively low and steady CBOD loadings, while the latter form of sloughing appeared to be associated with relatively high and fluctuating CBOD loadings.

The data for the RBC biofilm concentrations, percent volatile matter, and percent nitrogen are presented in Table 11. These data show that the highest biofilm concentrations were associated with the higher pH levels. Only the addition of calcium hydroxide resulted in an increase in inert matter entrained within both the first and second stage biofilms and a significant increase in effluent suspended solids. Sodium hydroxide, sodium carbonate, and sodium bicarbonate additions did not affect the biofilm

Table 11. Mean Biofilm Concentrations, Percent Volatile Matter, and Percent Nitrogen in the RBC Disc Biofilm of the Alkaline Chemical Addition Study^a

RBC- Stage	Alkaline Chemical	Stage pH	Biofilm mg/cm ²	Volatile %	Biofilm Nitrogen ^b %
1-1	NaOH	8.5	2.45	86	5.7
2-1	NaHCO ₃	7.5	2.08	86	5.7
3-1	Na ₂ CO ₃	8.4	2.51	87	7.5
4-1	Ca(OH) ₂	8.5	3.03	66	7.0
5-1	Control	7.0	1.57	87	7.6
1-2	--	7.9	0.97	89	5.4
2-2	--	7.7	0.84	90	7.6
3-2	--	8.0	0.96	89	6.4
4-2	--	7.9	1.88	70	6.0
5-2	--	6.9	0.87	90	6.8

^aSamples taken at weekly intervals from Day 36 to Day 75

^bNitrogen percentages are based upon weekly samples from Day 23 to Day 75.

volatile content; however, a slight increase in volatile content in all the second stage biofilms was noted. The addition of sodium hydroxide, sodium bicarbonate, and sodium carbonate caused only a slight increase of from 1 to 3 mg/l in the suspended solids in the RBC effluents; whereas the use of calcium hydroxide increased the effluent suspended

solids by approximately 20 mg/l. The observed increase in the calcium hydroxide RBC biofilm inert content as well as the increase in suspended solids is attributed to the reaction between the calcium hydroxide and the carbonic acid or carbon dioxide in the wastewater to form calcium carbonate.

The populations of ammonia-oxidizing, nitrite-oxidizing, and heterotrophic bacteria were monitored for both stages of each RBC system. Figures 8 and 9 present graphically the data on the relative geometric mean bacteria populations per unit of disc area and per unit weight of dry volatile biofilm for the stages of each RBC system from Day 36 to Day 74. This time period corresponds to the same period over which the relative nitrification rates are compared in Tables 8 and 9. The populations of all three groups of bacteria per unit area were greater for the first stages of the three high pH, high performance systems than for the first stages of sodium bicarbonate and control RBC systems. The first stages of the former were maintained at pH 8.4 to pH 8.5 while the latter were maintained at pH 7.5 and 7.0 for the sodium bicarbonate and control RBC systems, respectively. In the second stages, where there was less CBOD, less disc biofilm, and no pH control, the population differences were not as dramatic. The ratios of the populations for the three groups of bacteria for both stages of each RBC system are presented in Table 12.

Results of this research effort had indicated throughout the various phases that heterotrophic activity and biofilm development were enhanced under elevated pH conditions. In order to provide additional information regarding this observation, approximately 400 cm² of new disc material was added to the first stage discs of the control (pH 7.0), the sodium bicarbonate (pH 7.5), and the sodium hydroxide (pH 8.5) RBC systems on Day 62. The development of biofilm and the establishment of heterotrophic populations on these discs were monitored through Day 77. The resulting data are presented in Figures 10 and 11. The data demonstrated that both the biofilm and the heterotrophic activity developed more rapidly as pH increased from pH 7.0 to pH 8.5. During this 15-day test period, the influent CBOD (soluble and inhibited) concentration was approximately 8 mg/l. However, significantly greater amounts of CBOD, if present, may overshadow the more subtle influence of pH.

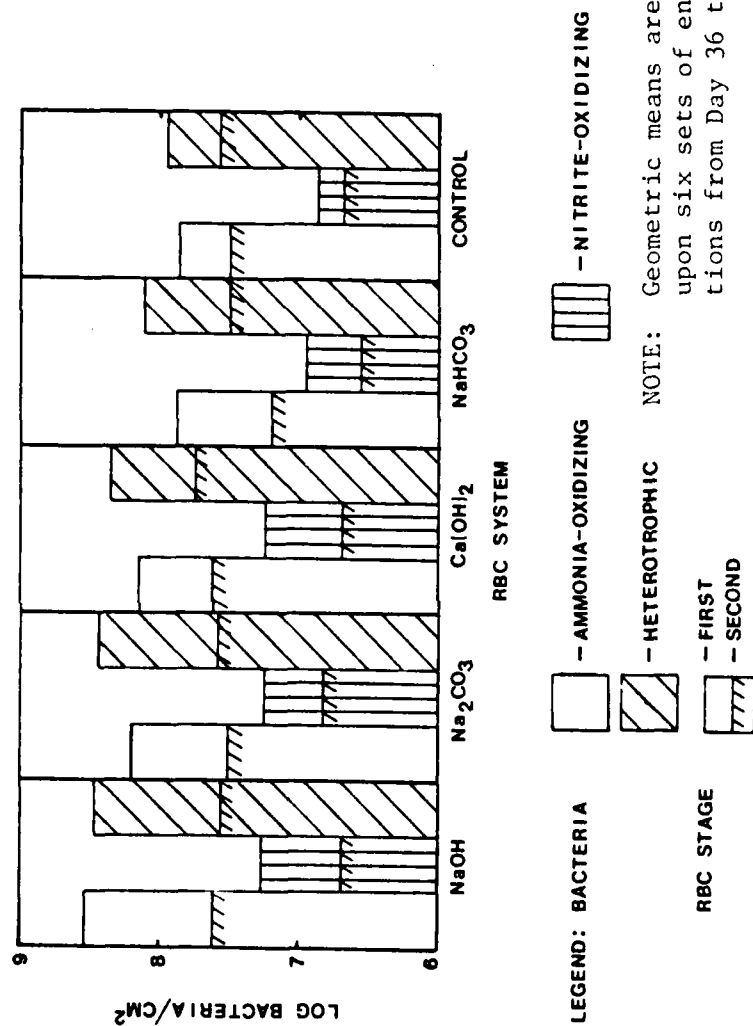


Figure 8. Relative Geometric Mean Populations of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria on a Unit Disc Area Basis for the Stages of the RBC Systems of the Alkaline Chemical Addition Study

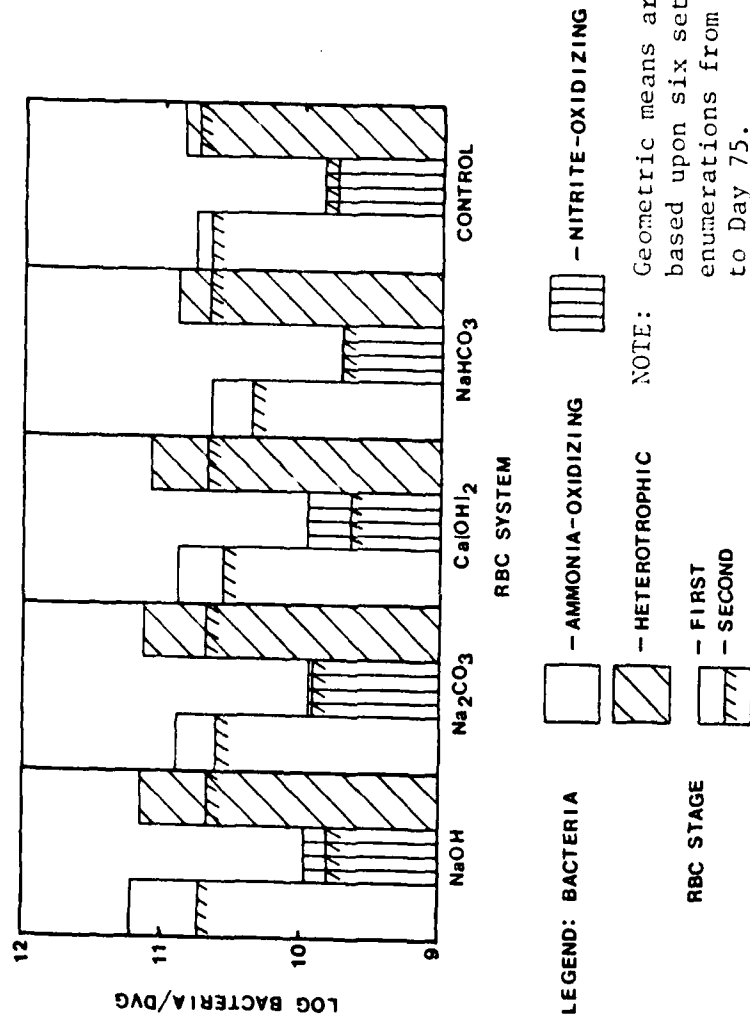


Figure 9. Relative Geometric Mean Populations of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria on a Unit Weight of Dry Volatile Biofilm Basis for the Stages of the RBC Systems of the Alkaline Chemical Addition Study

Table 12. Ratio of Heterotrophic:Ammonia-Oxidizing:
Nitrite-Oxidizing Bacteria for the RBC Stages
of the Alkaline Chemical Addition Study^a

RBC	Heterotrophs:Ammonia-Oxidizers:Nitrite-Oxidizers	
	Stage 1	Stage 2
NaOH	14 : 16 : 1	7.1 : 8.0 : 1
Na ₂ CO ₃	14 : 8.6 : 1	5.5 : 4.7 : 1
Ca(OH) ₂	12 : 7.8 : 1	11. : 7.9 : 1
NaHCO ₃	13 : 7.5 : 1	8.1 : 4.0 : 1
Control	11 : 9.4 : 1	7.3 : 6.1 : 1

^aRatios are based upon the geometric mean of 6 sets of samples taken at weekly intervals from Day 36 to Day 74.

A summary of the alkalinity destruction rates based upon data obtained from both continuous and batch operations is presented in Table 13. Except for the rate observed in the first stage of the calcium hydroxide RBC, all the alkalinity destruction rates were in the range of commonly accepted values. The unusually low alkalinity destruction rate observed in the bulk solution of the first stage of the calcium hydroxide RBC is attributed to the buildup of calcium carbonate within the biofilm which effectively neutralized some of the acid generated by the nitrifying bacteria within the biofilm. The net result was to reduce the overall amount of alkalinity destroyed in the bulk solution during the nitrification process.

SUMMARY AND CONCLUSIONS

This research examined the short and long-term effect of pH upon the nitrification of wastewater within RBC fixed film systems. In the long-term, the rate of nitrification

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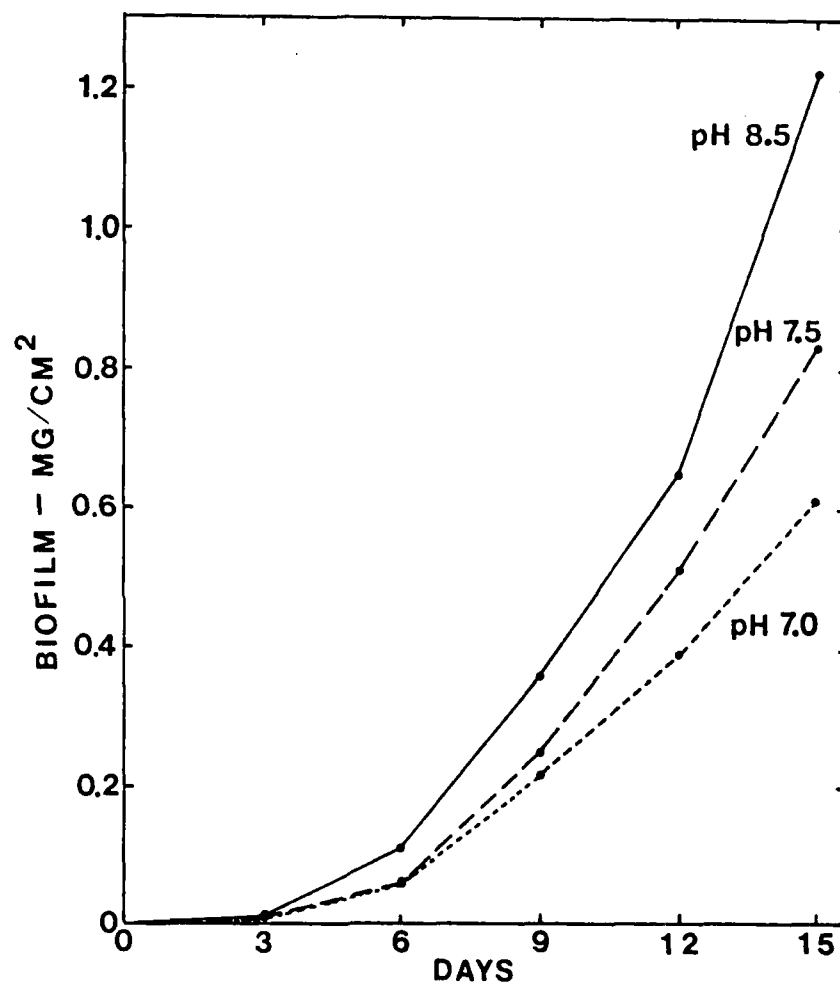


Figure 10. Relative RBC Biofilm Development under pH Conditions from pH 7.0 to pH 8.5

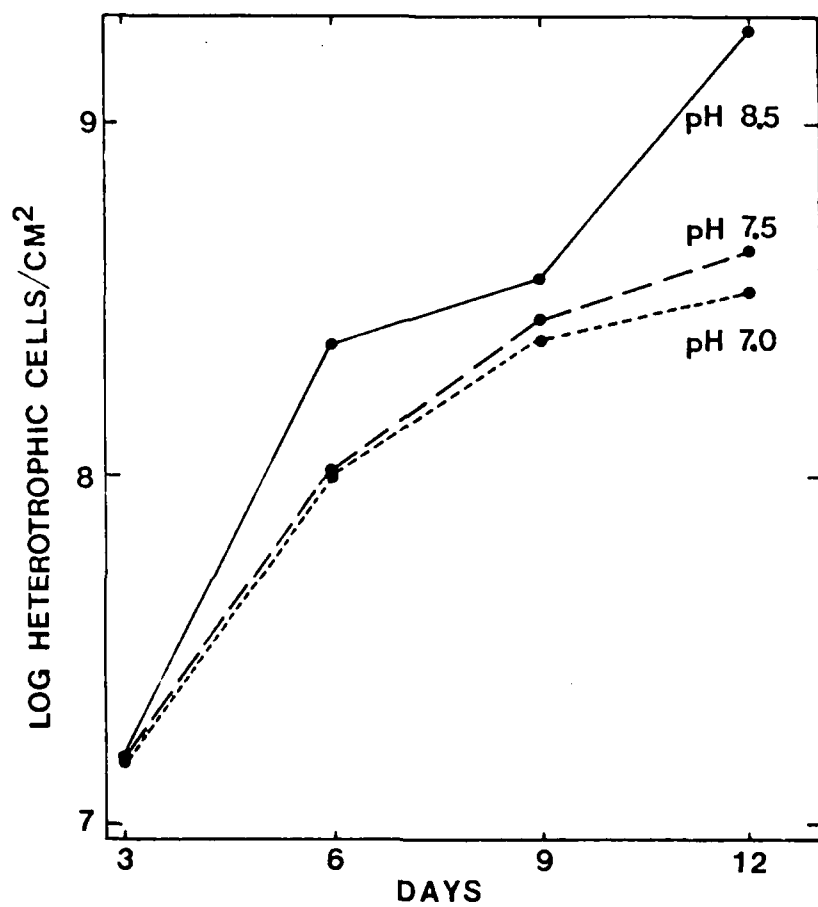


Figure 11. Relative RBC Heterotrophic Bacteria Growth Under pH Conditions from pH 7.0 to pH 8.5

Table 13. RBC Nitrification and Alkalinity Destruction
During the Alkaline Chemical Addition Study

RBC - Stage	Alkaline Chemical	Alkalinity Destruction (mg CaCO_3 /mg $\text{NH}_3\text{-N}$)	
		Continuous Operation ^a	Batch Operation
1-1	NaOH	--	6.2
2-1	NaHCO_3	--	6.8
3-1	Na_2CO_3	--	7.4
4-1	Ca(OH)_2	--	3.8
5-1	Control	7.4	6.6
1-2	--	6.1	--
2-2	--	7.7	--
3-2	--	7.9	--
4-2	--	7.0	--
5-2	--	7.2	--

^aBased upon data for the continuous operation from Day 38 to Day 75.

Within an RBC fixed film system was dependent upon pH. The rate of nitrification increased with increasing pH up to a maximum at pH 8.5. Approximately five weeks of operation were required to clearly observe these differences. The response of a nitrifying RBC system to short-term changes in pH was relatively constant from pH 7.0 to pH 8.5. Below pH 7.0, the adverse effect of pH becomes more pronounced.

However, the absolute level of nitrification was dictated by the biofilm's previous history of nitrification performance. RBC systems continued to nitrify at a relatively high rate after the pH had been reduced suddenly. The nitrogen balances for the various research phases revealed that the relative amount of nitrogen recovered for each RBC system generally was slightly less for the higher pH systems. This result was attributed to low level ammonia stripping as well as the loss of nitrate due to denitrification within the biofilm.

There was no significant difference in the performance of the 2-stage nitrifying RBC systems which received calcium hydroxide, sodium carbonate and sodium hydroxide. The performance levels of the sodium bicarbonate and the control RBC systems were 6 and 16 percent less, respectively, than those of the other three systems. The use of alkaline chemicals to maintain approximately pH 8.5 in the first stage of a 2-stage nitrifying RBC resulted in the removal of approximately 19 percent more ammonia than in the control RBC system. Except for the first stage of the RBC receiving calcium hydroxide for pH adjustment, the range of alkalinity destruction for all RBC systems in the alkaline chemical addition study was from 6.2 to 7.9 mg CaCO_3 /mg $\text{NH}_3\text{-N}$. This result was attributed to a neutralization capacity which developed within the RBC biofilm due to the entrainment of CaCO_3 . The production of significant amounts of inert material and suspended solids when calcium hydroxide is used, favors the use of sodium carbonate and sodium hydroxide when the nitrification is not followed by secondary clarification.

Higher levels of nitrification for the RBC systems were associated with greater disc biofilm uniformity. In all cases, except for the pH 8.8 RBC of the high pH study, the higher pH RBC systems maintained greater concentrations of volatile biofilm per unit of RBC disc area. The loss of biofilm from the RBC disc surface did not follow the traditionally accepted sloughing pattern. Biofilm did not slough from the disc surface outward. The dominant pattern of biofilm loss was from the biofilm surface inward. This loss was due to hydraulic shear at the biofilm surface. The RBC disc biofilm characteristics changed with time. The initial biofilm was uniform in texture and tan to bronze in color. The biofilm went through an aging process wherein the biofilm became darker and the texture became less uniform; the lower the pH, the less uniform the biofilm. The disc biofilm was affected greatly by low level changes

in CBOD. The maximum rates of nitrification for individual RBC stages were not associated with the maximum biofilm concentrations on the discs. Disc biofilm continued to develop after the individual RBC stages achieved their maximum rate of nitrification. The elevated pH RBC biofilms, which had enhanced nitrification capacities, had higher nitrifying bacterial populations than the lower pH RBC biofilms. The ammonia-oxidizing bacteria generally were favored over the nitrite-oxidizing bacteria with respect to increasing pH. Greater heterotrophic growth and more rapid biofilm development was observed to occur at elevated pH levels.

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